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Review

Historical perspectives on the impact of *n*-3 and *n*-6 nutrients on health

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ABSTRACT

Current public advice from the Food and Nutrition Board (FNB) about essential fatty acids (EFA) has limited quantitative details about three processes: (1) similar dynamics for *n*-3 linolenic and *n*-6 linoleic polyunsaturated fatty acids (PUFA) in maintaining 20- and 22-carbon *n*-3 and *n*-6 highly unsaturated fatty acids (HUFA) in tissues; (2) different dynamics for tissue *n*-3 and *n*-6 HUFA during formation and action of hormone-like eicosanoids; (3) simultaneous formation of non-esterified fatty acids (NEFA) and low density lipoprotein (LDL) from very low density lipoprotein (VLDL) formed from excess food energy and secreted by the liver.

This report reviews evidence that public health may benefit from advice to eat less *n*-6 nutrients, more *n*-3 nutrients and fewer calories per meal. Explicit data for linoleic acid fit an Estimated Average Requirement (EAR) near 0.1 percent of daily food energy (en%) meeting needs of half the individuals in a group, a Recommended Dietary Allowance (RDA) near 0.5 en% meeting needs of 97–98 percent of individuals, and a Tolerable Upper Intake Level (UL) near 2 en% having no likely risk of adverse health effects. Quantitative tools help design and monitor explicit interventions that could beneficially replace imprecise advice on "healthy foods" with explicit preventive nutrition.

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Abbreviations: AI, adequate intake is advised for 98% of people when RDA is not set; BMI, body mass index, reflects adiposity; Cal, kilocalorie, often used in nutrition discussions; CHD, coronary heart disease, esp. heart attacks; CVD, cardiovascular disease, includes CHD; DHA, docosahexaenoic acid, 22:6*n*-3; DPA, docosapentaenoic acid, 22:5*n*-3; EAR, estimated average requirement meets needs of 50% of people; EFA, essential fatty acids, have *n*-3 or *n*-6 structures and varied chain lengths and double bonds; en%, percent of daily food energy; ENHANCE, Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression trial; EPA, eicosapentaenoic acid, 20:5*n*-3; FNB, Food & Nutrition Board, for the National Research Council; HRA, health risk assessment, often the amount of a biomarker; HUFA, 20- and 22-carbon highly unsaturated fatty acids with 3 or more double bonds; IL-8, interleukin-8, an inflammatory cytokine; JUPITER, Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin trial; Km, Michaelis constant, describes a substrate level giving half-maximal activity; LDL, low density lipoproteins, formed from VLDL in plasma; LTB, leukotriene B; NEFA, non-esterified fatty acids; PDAY, Pathobiological Determinants of Atherosclerosis in Youth study; PUFA, polyunsaturated fatty acids, with varied chain lengths and two or more double bonds; RDA, recommended dietary allowance, meets needs of 98% of people; TNFα, tumor necrosis factor α, an inflammatory cytokine; UL, tolerable upper intake level, that has no likely risk of adverse health effects; USA, United States of America; USDA, United States Department of Agriculture; VLDL, very low density lipoproteins, secreted by liver.

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"All truths are easy to understand once they are discovered; the point is to discover them."

[– Galileo Galilei]

1. Public guidelines in the United States of America (USA)

The National Academy of Sciences was formed in 1863 to advise the federal government on scientific and technical matters, and in 1916, it organized the National Research Council to serve as its principal operating agency to provide services to the public and the scientific community. In 1940, the Council formed a Food and Nutrition Board (FNB) to study the safety and adequacy of the USA food supply; establish principles and guidelines for good nutrition; and provide authoritative judgment on the relationships among food intake, nutrition, and health maintenance and disease prevention (e.g., 1). At that time, estimates of essential nutrient efficacy were routinely made from dose–response studies and described in terms of an "*optimal*" amount needed per day. Several *n*-3 and *n*-6 essential fatty acids (EFA) were recognized from studies with laboratory animals, and many questions rose regarding possible mechanisms by which the nutrients maintained health.

The past 50 years brought recognition that dietary EFA accumulate as highly unsaturated fatty acids (HUFA) in tissues where they form hormone-like eicosanoids that act on selective receptors influencing physiological processes in nearly every tissue of the body. Competing metabolism by *n*-3 EFA can moderate actions of the *n*-6 EFA. The relative intakes of *n*-6 and *n*-3 nutrients create tissue HUFA proportions that create a propensity or predilection for more or less vigorous eicosanoid actions, respectively. Many effective pharmaceutical agents decrease excessive actions by *n*-6 eicosanoids. The benefits obtained from reducing *n*-6 mediator actions when using such agents give a new perspective on a possible tolerable upper intake level (UL) for *n*-6 nutrients.

The wide scope of harmful *n*-6 eicosanoid actions now known to occur requires careful discrimination between associated and causal mediators when advising the public about "optimal" intakes of *n*-3 and *n*-6 nutrients. Rigorous logic can avoid evidence-based misunderstandings. Despite repeated warnings that correlation is not evidence of causation and that a lack of evidence for an effect is not evidence for the lack of an effect [2], misleading advice can occur. Inadequate logic or neglect of certain evidence can impair the effectiveness of advice. A continuing unfolding of new scientific insights creates a continuing need to revise health advice to the public.

The following discussion examines quantitative evidence for actions of *n*-3 and *n*-6 essential fatty acids in the context of health maintenance and disease prevention. It notes examples in which current public advice might be re-phrased, and it describes simple arithmetic tools by which individuals may make informed choices of foods and monitor metabolic outcomes. The review moves from basic nutritional science through biomedical metabolic insights toward practical applications of that information for primary prevention of health disorders.

2. Essential fatty acids in human nutrition

In 1946, the FNB asked two pioneers in the study of essential fatty acids (EFA), Arild Hansen and George Burr, to review the current knowledge [3] about a possible human need for these nutrients first described in 1929 as essential for rats [4]. The researchers noted that very low amounts were needed, and "*the observation proving linoleic acid and arachidonic acid to be dietary essentials was not made until young laboratory animals had been maintained on diets extremely low in fat for relatively long periods of time. The likelihood that a human infant would subsist for a prolonged period on a diet practically devoid of fat, yet complete as regards other known dietary essentials, is practically nil.*"

Physiological signs used for quantitative assay of essential fatty acid actions in rats were (i) development of scaly skin and caudal necrosis, (ii) retardation of growth, (iii) increased water consumption and (iv) early death. These were prevented by feeding linoleic (18:2*n*-6), linolenic (18:3*n*-3), arachidonic (20:4*n*-6) and docosahexaenoic (22:6*n*-3) acids. Prophylactic doses of 20–25 mg of linoleic acid promoted good growth and reproduction of rats, and 50–100 mg daily seemed superior for curative treatments. For reasons not apparent now, the authors chose the highest content studied (100 mg for 10 g diet, i.e., 1%) as a "*minimum adequate daily intake*" of linoleic acid for a rat.

In 1946, with no clear evidence for a lack of essential fatty acids in humans, Hansen and Burr suggested that a "*required*" level of intake for humans might be 1 per cent of the diet based on the assumption that humans and rats have similar metabolic responses to these nutrients [3]. Their review concluded by noting that the greatest difficulty in studying quantitative requirements for humans was an inability to monitor accurately the status of essential fatty acids in blood and tissues. This problem was addressed first by spectrophotometric estimates of unsaturated fatty acid content and later by detailed, informative gas chromatographic analyses.

2.1. Evidence from infants

To look for clinical signs in humans, Hansen and his colleagues began an extensive clinical study of several hundred infants [5]. The chief criteria for selection were the parent's wish to cooperate and a normal neonate status of the infant. The babies included children of physicians and medical students plus children seen in Well Baby Clinics. Infants ate one of several different milk mixtures with different contents of linoleic acid. The group eating a milk mixture with only 0.04 percent of food energy (en%) as linoleic acid had unsatisfactory progress, and a fifth diet with 0.07 en% linoleate was added while the 0.04 en% diet was eventually terminated.

Signs for most of the infants fed 0.04 en% linoleate were "*frequent large stools and perianal irritation*". Also, skin alterations were discernible within weeks in the majority of these infants. In each instance when the milk mixture was changed to one containing more than 1 en% linoleic acid, the diarrhea stopped, the rash in the diaper region disappeared, the raw exuding areas cleared and the skin gradually returned to a normal soft velvety texture.

Rates of growth were similar and satisfactory for almost all of the infants who received 1.3%, 2.8% and 7.3% of calories as linoleic acid; whereas progress was unsatisfactory for many, but not all, of the infants with linoleic acid intakes below 0.1 en%. Following introduction of cereals to their diet, all infants had a gradual decrease in deficiency signs as well as increased circulating levels of dienoic acid in the blood. Signs of deficiency disappeared promptly whenever linoleic acid provided 1% or more of food energy.

The early decision to add a fifth diet with 0.07 en% linoleic acid gave important data in the very narrow range sensitive to dietary linoleate efficacy. In the first 3 months, unwanted dermal signs appeared in 100% infants receiving 0.04 en% linoleate, but in only 40% infants receiving 0.07 en%. Eating less than 0.1 en% prevented signs of essential fatty acid deficiency in half of the babies. This result acquires greater significance in the discussion in Section 9. Results gathered over four years with 428 infants confirmed that linoleic acid is an essential nutrient for human infants [6], and the lack thereof was described as EFA deficiency.

3. Converting dietary essential fatty acids (EFA) into tissue highly unsaturated fatty acids (HUFA)

Holman [7] used an alkaline isomerization spectrophotometric method to measure very sensitive metabolic responses of endogenous polyunsaturated fatty acids in rats to dietary linoleate levels between zero and 1 en%. By 1960, research had shown that metabolism of both dietary 18-carbon polyunsaturated fatty acids (PUFA), linoleic and linolenic acid, led to formation and accumulation of 20- and 22-carbon highly unsaturated fatty acids (HUFA) in tissues of rats and humans. The amount of trienoic acid formed from the monoenoic oleic acid fell as the tetraenoic arachidonic acid formed from linoleic acid rose in response to diets, and the different observed proportions of accumulated trienoic and tetraenoic HUFA were similar in plasma, erythrocytes and heart tissue.

Only two of seven diets tested with rats produced dermal scores significantly above zero. Those diets had 0 and 0.14 en% linoleic acid. In contrast, no significant dermal score indicating an EFA deficiency occurred with diets that had 0.56, 1.12, 4.48, 5.1 and 20.2 en% linoleate. Holman noted that the tetraene value rose to a maximum value when dietary linoleic acid rose from 0 to 1 en%. He suggested that a "*break point*" near 1 en% linoleate (corresponding to a triene/tetraene ratio of 0.4) marked a transition between "*inadequate*" and "*adequate*" EFA status (even though no deficiency signs occurred for rats eating 0.56 en%).

Spectrophotometric measurements of the di-, tri- and tetraenoic acid levels in the blood of the 428 infants studied by Hansen et al. [6] showed similar metabolic biomarker responses to linoleic acid by rats and humans [8]. Data for the two low linoleate intakes of 0.04 and 0.07 en% differed greatly from the data for 1.3, 2.8 and 7.3 en% linoleate. Infants eating less than 0.1 en% had many measures of deficiency, whereas those eating more than 1 en% had very few measures of deficiency.

With only five levels of intake to examine, a "*biphasic*" hyperbolic curve of EFA status could not be clearly defined, although the metabolic response of the biomarkers closely resembled the more detailed results observed with rats. The sensitive increase of tissue tetraenes to small additions of dietary linoleate was not seen with dietary intakes above 1 en%. The authors regarded results from feeding linoleate at less than 0.1 per cent of calories to not be a "*practical problem*" associated with a "*practical nutritional range*" [8] even though the important midpoint for response to dietary linoleate was below 0.1 en%. They proposed that about 1 per cent of food calories was a "*minimal linoleate requirement*" for the human infant.

3.1. Gas chromatography gives explicit values

Gas chromatography was used next in a comprehensive study of the effect of dose level of EFA upon the fatty acid composition of rat tissues [9]. The researchers fed many groups with less than 1 en% of essential fatty acids because previous experiments had shown the most dramatic dose–response changes in this region. Diets included 0.01, 0.02, 0.05, 0.10, 0.18, 0.32 and 0.61 en% linoleic acid and 0.01, 0.02, 0.04, 0.08, 0.18, 0.32 and 0.61 en% linolenic acid. Low levels of both acids were similarly effective in promoting growth of rats. The hyperbolic rise in the proportions of *n*-3 or *n*-6 HUFA (see graphical array in Fig. 4 in Ref. [10]) was very sensitive to small dietary increments, fitting a mid-point response with intakes near 0.1 en% and "*plateauing*" with dietary intakes above 1 en%.

A graph of lower accumulated 20:3*n*-9 with higher intakes of EFA (see Fig. 4 in Ref. [10]) showed that both the *n*-6 linoleic and *n*-3 linolenic acids had similar high efficacies in competing against endogenous *n*-9 oleic acid during elongation and desaturation metabolic steps. The metabolic efficacy and EFA activity for both EFA fit a mid-point near 0.1% dietary food energy. Further study of the 20:3 fraction showed that it contained primarily 20:3*n*-9 formed from endogenous oleate (18:1*n*-9) with small amounts of 20:3*n*-7 formed from palmitoleate (16:1*n*-7). More recent detailed results [11] confirmed that metabolic dynamics for dietary *n*-3 and *n*-6 PUFA are very similar during conversion to the corresponding HUFA. Approximately 78% of both EFA was catabolized or excreted, 16–18% of both was accumulated unchanged in tissues, and about 6% of 18:3*n*-3 and 2.6% of 18:2*n*-6 was accumulated as HUFA in tissues [11]. The similar metabolic dynamics for the *n*-3 and *n*-6 nutrients make their relative dietary supply an important controlling factor for the relative *n*-3 and *n*-6 proportions that accumulate in tissue HUFA (see Section 9). The results also illustrate how pivotal the diet with 0.07en% linoleic [5,6] was for interpreting EFA status and informing us about the quantitative need for small amounts of EFA in humans.

A detailed gas chromatographic study of the competitive metabolic interactions between linoleate and linolenate [12] used many dietary levels in the narrow range near the onset of EFA deficiency comparable with those used when linoleate and linolenate were fed singly [9]. Although mild dermatitis occurred with some rats having linoleate intakes near 0.08 en%, no severe signs of deficiency were evident with rats eating more than 0.3 en% linoleate. Again, the evidence confirmed that metabolic and physiological efficacy is similar for rats and humans as suggested in the 1946 report to the FNB [3]. The proportions among different 20- and 22-carbon HUFA were sensitive indicators of the relative supplies of *n*-3 and *n*-6 EFA. This study [12] also described another trienoic acid, 20:3*n*-6, which was derived from linoleate and accumulated in tissues. The presence of several forms of trienoic acids in tissues made continued use of general triene/tetraene ratios for estimating EFA status an imprecise and over-simplified exercise and gas chromatographic assays of HUFA are now a standard measure of EFA status.

4. A sharpened focus on EAR and RDA

Terminology currently used by the Food and Nutrition Board (FNB) to convey aspects of nutrient requirements is in a set of

Dietary Reference Intakes [1] that expand and replace previously published "*Recommended Dietary Allowances*" and "*Recommended Nutrient Intakes*". The new dimensions include an Estimated Average Requirement (EAR), an intake that meets the estimated nutrient needs of half the individuals in a group. A higher intake that meets the nutrient need of almost all (97–98 percent) individuals in a group is called a Recommended Dietary Allowance (RDA). By 1963, the sensitive hyperbolic response of tissue biomarkers and clinical signs to dietary essential fatty acids recognized after the 1946 FNB request for clarification [3] fit an EAR slightly below 0.1 en% and a RDA near 0.5 en%. Two further clarifications of these values are noted below.

Collins et al. [13] described a patient who developed a dry scaly rash on his face and chest after 60 or 70 days of fat-free intravenous therapy. Gas chromatographic assays showed that the proportion of the triene 20:3*n*-9 in plasma HUFA had been more than that for the tetraene 20:4*n*-6 for weeks before the rash was evident. These proportions reversed and the rash disappeared within days after administering an emulsion of soybean oil (43% linoleic and 6.5% linolenic acid). After two weeks, the EFA infusion was stopped, and the 20:3*n*-9 level gradually became greater than that for 20:4*n*-6 for weeks before the skin rash recurred and the fat infusion was resumed. Following continued EFA infusion, the rash disappeared and remained absent.

This explicit longitudinal evidence of accumulated HUFA proportions sharpens Holman's earlier interpretation [7,8] that a triene/tetraene ratio of 0.4 seemed to indicate a borderline EFA deficiency. More precise gas chromatographic measurements indicate that the borderline likely occurs after the proportions of the *n*-9 acid is 50% or greater in blood HUFA for some weeks. Put in other terms, the transition from EFA deficiency occurred when the %*n*-6 in HUFA rose above 50% [13]. This biomarker for a transition from EFA deficiency is obtained with dietary linoleate intakes between 0.1 and 0.5 en%. Section 9 of this review examines a paradoxical aspect of this narrow range in more detail.

Cuthbertson [14] noted a paradox in the advice [6] that a "*minimal linoleate requirement*" for the human infant was "*about 1 per cent of food calories*". The absence of any clinical signs of EFA deficiency in the U.K. contrasted with evidence that the majority of baby foods in the U.K. provided only about 0.6 en%. If the minimum EFA dietary requirement was, indeed, as high as 1.0 en%, deficiency signs should have been more common than they were. Thus, he concluded that the "*minimum EFA requirement*" had been set too high, and that it was likely less than 0.5% of food energy. The absence of severe signs of EFA deficiency in rats eating linoleate at 0.56 en% [7] and more than 0.3 en% [12] again confirm the very similar metabolic dynamics and very low level of dietary need for this essential fatty acid in rats and humans suggested in the 1946 report to the FNB [3].

5. Essential fatty acid actions and molecular medicine

Quantitative dose–response data reported in 1963 [6,9,12] made it clear that humans (and rats) require dietary polyunsaturated fatty acids (PUFA) which are metabolized to longer highly unsaturated fatty acids (HUFA) that accumulate in tissues. To use biomarkers of EFA status effectively for disease prevention, the public needs valid measures of how these essential nutrients mediate health maintenance and disease prevention. In 1963, the stage was set to discover specific molecular events by which essential fatty acids exert their impact on human health.

In the following year, 1964, Bergström and colleagues [15] made a large step forward as they began describing the metabolic conversion of tissue HUFA to hormone-like compounds called prostaglandins. Over the next two decades, the list of newly discovered EFA-derived bioactive molecules and their important physiological and pathological processes grew rapidly and were recognized by the 1982 Nobel Award in Physiology or Medicine.

Molecular mechanisms mediating the processes of inflammation, thrombosis and bronchoconstriction became recognized, and researchers developed many new pharmaceutical agents to decrease excessive formation and action of specific bioactive agents formed from the major tissue *n*-6 HUFA, arachidonic acid. Research showed that aspirin-like drugs had beneficial anti-inflammatory, anti-thrombotic and analgesic actions by slowing the formation of *n*-6 prostaglandins from arachidonate.

However, the newly recognized bioactive molecules did not give ready answers for how essential fatty acids prevent physiological signs of increased water consumption, development of scaly skin and retarded growth. Also, the different effectiveness of *n*-3 and *n*-6 nutrients to prevent these signs during conditions of water restriction [16] remained poorly understood. Insight on how such phenomena connect to dietary EFA was delayed until the recent brilliant work on skin lipids by Alan Brash and coworkers [17]. They hypothesize [18] that two epidermal lipoxygenases, 12R-LOX and eLOX3 convert the linoleate ester in complex ceramides into hepoxilins that act as a signal for further covalent binding of the glycolipids to corneocyte proteins which forms a competent skin barrier. The *n*-6 intermediates appear to be more effective than *n*-3 forms for at least one of these steps.

Instead of explaining how *n*-3 and *n*-6 nutrients prevent classical signs of EFA deficiency, research from 1964 to 2004 provided a stunningly expanded network of EFA-based physiological and pathophysiological mediators that act on selective receptors that occur on nearly every cell and tissue in the human body. New research showed increasingly sophisticated signaling networks by which mediators derived from dietary PUFA and tissue HUFA regulate cytokines and chemokines and affect human health. Paradoxically, a principal goal for successful new drug development was to diminish excessive actions of the essential *n*-6 HUFA, arachidonate. The situation suggests that the average dietary supply of *n*-6 essential nutrients may be more than needed for good health.

Some of the new drugs (like the long-used aspirin) have a fairly narrow therapeutic window between efficacy and harm, and they require careful monitoring to avoid inhibition of beneficial *n*-6 mediated events. As more EFA-based mediators of chronic diseases became known, billions of dollars went into developing and marketing agents that successfully lowered unwanted excessive formation and action of *n*-6 prostaglandins, leukotrienes and thromboxane (while hopefully allowing needed *n*-6 mediator actions to remain intact).

Knowledge grew rapidly about how tissue arachidonate mobilized by phospholipase action is converted to specific bioactive lipids that mediate major aspects of cardiovascular disease: inflammatory plaques and thrombosis. The biomedical community now knows a valid sequence of molecular events by which dietary EFA cause tissue HUFA proportions that affect health maintenance and disease prevention. The wide scope of unwanted disorders known to be made worse by over-actions of *n*-6 mediators prompts serious questions about an acceptable range of values for *n*-6 nutrient intake and *n*-6 tissue status. What tolerable upper intake level for *n*-6 nutrients is likely to have no risk of adverse health effects?

In this regard, the corresponding *n*-3 HUFA are less active in forming prostaglandins [19], and they diminish arachidonate-mediated processes in animal models of stroke [20] and heart attack [21]. This knowledge raised hope that a lowered intake of *n*-6 EFA precursors and increased intake of competing *n*-3 precursors might moderate excessive unwanted actions of linoleate-based mediators in humans. The idea stimulated a hypothesis 30 years ago that deliberate dietary changes might provide primary prevention of several serious diseases [22].

5.1. An early clue to a narrow therapeutic window

An effort to decrease inflammatory disorders in rats by feeding a very low level of dietary linoleic acid gave a HUFA status of 30% *n*-6 in HUFA (with 70% *n*-9 in HUFA). This condition decreased macrophage adherence to about half [23]. Interestingly, decreasing the inflammatory process needed low proportions of *n*-6 HUFA similar to those reported by Collins et al. [13] to cause signs of EFA deficiency. This result suggests that in the absence of counter-balancing *n*-3 nutrients, dietary *n*-6 linoleic acid may have a very narrow (or non-existent) therapeutic window. As a result, questions of ethics plus the difficulty of arranging such low dietary intakes for humans (near the EAR of 0.1 en%) shifted interest toward eating more *n*-3 nutrients to lower competitively the proportion of *n*-6 HUFA while they raised the proportion of *n*-3 HUFA in total tissue HUFA. This approach acquires more significance in Section 9 of this review. A unique, detailed study of enzymes and receptors showed many (but not all) *n*-3 analogs are active, but less potent than the corresponding *n*-6 mediators in promoting receptor-mediated processes [24]. It became apparent that changes in the balance between moderate actions of *n*-3 mediators and vigorous actions of *n*-6 mediators can provide a transition from healthy physiology to pathophysiology. Replacing tissue *n*-6 HUFA with *n*-3 HUFA can widen the therapeutic window for dietary *n*-6 linoleate.

The competitive hyperbolic relationship that tissue biomarkers of EFA status have with dietary supplies of *n*-3 and *n*-6 EFA [9,12] was confirmed quantitatively with rats [25] and extended to humans [26]. The sensitive response of tissue HUFA status to PUFA intakes below 0.5 en% contrasts with the relatively insensitive response to dietary amounts above 2 en%. Such behavior is typical for metabolism catalyzed by saturable active sites and characterized by the 100-year old empirical Michaelis–Menten relationship.

The empirical Michaelis–Menten constant, Km, is conceptually analogous to an EAR for essential nutrients. Empirical constants in a quantitative empirical equation fitting data for humans and rats were near 0.05 en% for linoleic and linolenic acids [26]. Thus, they confirm the value near 0.1 en% from earlier studies with rats [7] and humans [4]. The equation [26,27] predicts the impact of dietary *n*-3 and *n*-6 nutrients on tissue *n*-3 and *n*-6 HUFA. It predicts successfully the impact of dietary essential fatty acids on tissue HUFA for data from 34 published studies of nearly 4000 people in 92 groups from 11 different countries [28].

6. Insights from cross-cultural comparisons

Epidemiologists have long known that ischemic heart disease was less prevalent for Mediterranean populations than for Americans while being more prevalent than for Japanese [22,29]. These cross-cultural differences are associated with different ethnic lifestyles and food choices that include food energy density and fat in the diet. Higher heart disease incidence for Japanese living in the USA [30] and rising rates following increased "Westernizing" of traditional diets [31] indicate that different food choices and lifestyles were a more likely cause than genetics. Traditional populations seem to unknowingly maintain a form of food-based primary prevention of cardiovascular disease, CVD. In describing the strong association of CVD with higher dietary fat intake for 21 population groups [29], Keys urged extensive research on "*the role of dietary fat in atherogenesis and thrombogenesis*". He noted that the percent of dietary food energy as fat (i.e., food energy density) was strongly associated with blood cholesterol levels and CVD incidence. The stage was set to discover specific molecular events by which food energy exerts its negative impact on human health.

Soon thereafter, the 1964 Nobel Award recognized intricate molecular pathways by which food energy intake causes the liver to form and secrete cholesterol and triacylglycerols in the form of very low density lipoproteins (VLDL). This information made cholesterol and triacylglycerol levels in blood credible biomarkers for the intake of food energy density. However, to use these two biomarkers as valid measures to monitor disease prevention, the public needs valid measures of how food energy density might act through these biomarkers to cause disease and death.

Keys hypothesized "the more common fats of the American diet, when eaten in large amounts as is often the case in the United States, may contribute to the production of relative hypercholesterolemia and so to atherogenesis" [29]. This cholesterol-centered hypothesis contrasted with earlier evidence [32] of Alaskan Eskimos eating much cholesterol and having high serum cholesterol with an almost total absence of cardiovascular-renal diseases. Wilber and Levine [32] regarded "the causative role of serum cholesterol in development of atherosclerosis to be somewhat dubious".

Although Keys disparaged [29] the evidence from Eskimos that contradicted his idea, subsequent data on the EFA status of Arctic people [33] eventually proved pivotal in alerting the public to important differences between *n*-3 and *n*-6 EFA actions in mediating cardiovascular disease. To better define preventable mediators that link cardiovascular disease to food energy density and fat in the diet, Keys recruited collaborators for the "*Seven Countries Study*", a large, long-term cross-cultural study of cardiovascular disease associated with different ethnic lifestyles and food choices [34].

6.1. Insight relating cholesterol and highly unsaturated fatty acid (HUFA) balance

A 25-year prospective follow-up of the Seven Countries Study [35] showed that absolute mortality rates for coronary heart disease (CHD) differed widely for a given level of the blood cholesterol biomarker for high food energy density. The authors of the 1995 report [35] suggested dietary factors that affect inflammatory processes and thrombosis were "*of great importance*". Those processes have explicit mediators made from tissue *n*-6 HUFA. For six mixed population groups in the study [35], biomarker levels for elevated food energy density (blood cholesterol) were associated with risk of death from CHD in the USA and Northern Europe. However, they

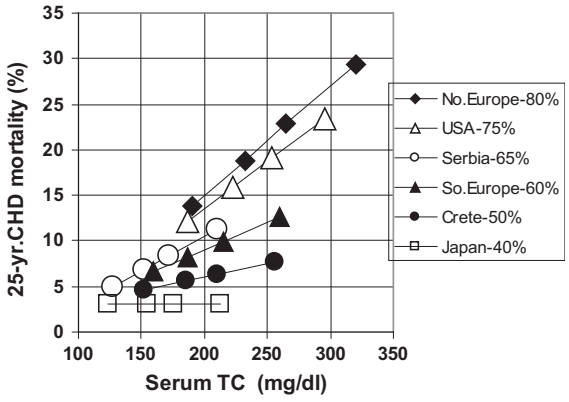

Fig. 1. Relating tissue HUFA balance with blood cholesterol and heart attacks. Results from the 25-year follow-up in the Seven Countries Study [35] were discussed in an earlier review [10] which noted that "*Food energy imbalances which elevate blood cholesterol may be fatal only to the degree that omega-6 (n-6) exceeds omega-3 (n-3) in tissue HUFA. Such evidence raises questions about the hypothesis that blood cholesterol levels cause CHD.*" Northern Europe and Southern Europe have abbreviations "No." and "So.", respectively. The Figure is reprinted with permission of the publisher.

had no clear association with CHD risk in Japan [35,36]. In fact, a recent study of 173,539 Japanese men and women showed slightly lower mortality with higher blood cholesterol levels [37]. Such evidence (see Fig. 1) raises questions about the hypothesis that blood cholesterol levels cause CHD.

In contrast to the cholesterol biomarker for food energy, tissue biomarkers for *n*-3 and *n*-6 EFA status are strongly associated with the severity of CHD in many diverse groups [38]. People with more than 50% *n*-6 in tissue HUFA (and less than 50% *n*-3 in HUFA) have a greater risk for CHD mortality than those with less than 50% *n*-6 in HUFA. Values for tissue HUFA status of different ethnic groups world-wide range from 32% to 87% *n*-6 in HUFA ([34], see also Table 2 in [10]). Estimates of the likely %*n*-6 in HUFA for the six populations in the 25-year follow-up [35] gave an empirical model that predicted the different observed slopes of absolute mortality vs. serum cholesterol for all of the subject groups (see Fig. 1). The balance between *n*-3 and *n*-6 in tissue HUFA seems a useful value for health risk assessment (HRA).

Associations among death, blood cholesterol and the HRA value of %*n*-6 in HUFA prompted the hypothesis that high food energy intakes which increase the formation and secretion of cholesterol and triacylglycerol may NOT lead to death when tissues have equal proportions of *n*-3 and *n*-6 HUFA [10]. The food energy biomarker (blood cholesterol) appears to predict CHD risk ONLY to the extent that the proportions of pro-inflammatory and pro-thrombotic *n*-6 EFA (%*n*-6 in HUFA) exceeds those of the complementary anti-inflammatory and anti-thrombotic *n*-3 EFA (%*n*-3 in HUFA). A similar situation is evident in chronological associations of the likely %*n*-6 in HUFA and the transition from benign to fatal prostatic hyperplasia [39]. Much evidence suggests that some populations unknowingly maintain a degree of primary prevention of chronic cardiovascular and immune-inflammatory disorders by eating foods that balance the *n*-3 and *n*-6 HUFA in their tissues [10,22,38,40].

To design diets that develop a desired balance of tissue HUFA, the empirical predictive equation [27] was placed within a simple spreadsheet [41]. The spreadsheet estimates that eating BOTH *n*-6 linoleate and *n*-3 linolenate at 0.5 en% (which fits the definition of their RDA and suppresses accumulation of *n*-7 and *n*-9 HUFA) would likely maintain both *n*-3 and *n*-6 HUFA at a level near 50%. A similar outcome is predicted when BOTH acids are ingested together at higher levels of 1, 2, 4 or 7 en%. However, continued intake of linoleate near 6.8 en% with linolenate near 0.8 en% predicts a imbalanced value near 79% *n*-6 in HUFA. This proportion is often observed in simple gas chromatographic analyses of whole blood in Americans [10,38,40].

The USDA Nutrient Database gives fatty acids in foods to use in calculating an Omega 3-6 Balance Score	Fatty acid	# 1 weight percent	# 2 weight percent	Finger-tip Assay gives fatty acids in blood to give the % n-6 in HUFA
	14:0	1.5	1.4	
	14:1	0.4	1.0	
	15:0	0.7	2.2	
	16:0	20.5	22.1	
	16:1ω7	2.1	3.3	
	18:0	7.7	6.1	
	18:1ω9	16.8	15.4	
linoleic	18:2ω6	27.2	23.5	
γ-linolenic	18:3ω6	0.7	0.3	
α-linolenic	18:3ω3	0.6	0.6	
stearidonic	18:4ω3	0.3	0.1	
	20:0	0.1	0.1	
	20:1ω7	0.1	0.0	
	20:2ω6	0.3	0.1	
	20:3ω9	0.1	0.0	eicosatrienoic
dihomo-γ-linolenic	20:3ω6	1.6	1.1	dihomo-γ-linolenic
arachidonic	20:4ω6	12.9	9.1	arachidonic
eicosapentaenoic	20:5ω3	0.8	4.7	eicosapentaenoic
	22:0	0.3	0.5	
	22:1ω9	0.4	0.4	
docosatetraenoic	22:4ω6	1.3	0.5	docosatetraenoic
docosapentaenoic	22:5ω6	0.3	0.2	docosapentaenoic
docosapentaenoic	22:5ω3	1.6	2.0	docosapentaenoic
docosahexaenoic	22:6ω3	1.6	4.7	docosahexaenoic
	24:1	0.1	0.2	
	Saturates	30.8	32.4	
	Monoene	17.7	17.0	
	HUFA	20.5	22.6	
	%n-3inHUFA	21	51	
	%n-6inHUFA	79	49	

Fig. 2. Gas chromatographic assays describe EFA balance in foods and tissues. Two examples of data from finger-tip blood-spot assays illustrate different results seen for individuals with different food habits. Eight HUFA shown on the right are combined into one health risk assessment (HRA) value of %*n*-6 in HUFA. For each food item, fatty acid contents were obtained from the USDA Nutrient Database [48], and mg/Cal values for the eleven different *n*-3 and *n*-6 fatty acids noted on the left were combined and expressed as a single numerical value, the Omega 3–6 Balance Score [49], to describe the likely impact of each food item on the HRA value.

7. Arithmetic for essential fatty acid (EFA) status in health and disease

A 2008 critique of paradoxes in advice on dietary lipids [10] described a connected causal chain of molecular events by which an imbalance of *n*-3 and *n*-6 EFA in foods creates a propensity or predilection for many chronic inflammatory and cardiovascular disorders. The connected events indicate that the easily measured proportion of *n*-3 or *n*-6 in HUFA [42] which monitors average dietary intakes can also be a valid surrogate for eventual clinical endpoints of saved lives or prevented disease. The transport and exchange of HUFA among tissue lipids is relatively indiscriminate with regard to the *n*-3 and *n*-6 structure, making relative proportions of *n*-3 and *n*-6 acids in HUFA fairly similar for blood, plasma, erythrocytes, or other tissues [25,42,43]. As a result, the percent of *n*-6 in the HUFA of whole blood is a valuable health risk assessment (HRA) measure for preventive medicine. Such surrogates are important cost-saving tools when large, expensive, long-term clinical trials need thousands of subjects for an otherwise long period of time to provide sufficient statistical power [44]. Measured HRA values clearly inform people of their status so they can voluntarily alter their food choices and HRA status.

To design menu plans that alter tissue HUFA balance for primary prevention of cardiovascular disease [45,46], a personalized, interactive software program [47] used the predictive relationship [27,41] with data from the USDA Nutrient Database [48] to develop explicit food choices that arrange *n*-3 and *n*-6 EFA intakes to produce whatever tissue proportion of *n*-3 and *n*-6 HUFA is desired.

For example, daily menu plans with different intakes of *n*-3 and *n*-6 EFA gave a likely tissue *n*-6 HUFA balance of 91%, 71%, 63%, 50%, 35%, 26% and 15% *n*-6 in HUFA (see Chapter 19, Ref. [45]). Daily menu plans stored in the program files [47] can be retrieved and modified to fit each individual's personal taste preferences and aversion to risk. To help people recognize more quickly the food items likely to have a desired impact on tissue HUFA balance, the mg/Cal values for eleven different *n*-3 and *n*-6 fatty acids in each food item (Fig. 2) were combined and expressed as an Omega 3–6 Balance Score [49]. In contrast to the 1992 empirical relationship for dietary EFA forming tissue HUFA [26], the Balance Score uses mathematical differences for four groups of 18-carbon *n*-3 and *n*-6 nutrients and 20- and 22-carbon *n*-3 and *n*-6 nutrients. It also includes a factor of 7 [49] to accommodate the more effective accumulation of the dietary HUFA into tissue HUFA as it predicts impacts related linearly to those predicted by the interactive software [47]. Foods with a positive score will increase the proportion of *n*-3 in tissue HUFA and those with a negative score will increase the proportion of *n*-6 in tissue HUFA.

Table 1
A simplified example of using Omega 3–6 Balance Scores to choose a daily menu plan. Eating 100 calories of each item gives an overall average balance of +2.

Selected food items	Omega 3–6 balance score
Cereals, ready-to-eat	−2
Milk, non-fat, fluid	0
Orange juice, raw	0
Potatoes, Russet	0
Finfish, salmon	+40
Broccoli, boiled	+3
Collards, boiled	+2
Cauliflower, boiled	+5
Pinto beans, cooked	0
Spinach, boiled	+1
Snap green beans	+1
Chicken, light meat	−5
Cheese, parmesan	0
Egg, whole, cooked	−12
Tea, sweetened	0
Bananas, raw	0
Ice cream, vanilla	−1
Average score of 19 items	+2

The calorie-weighted average score for the foods eaten in a day relates directly to the proportions of *n*-3 and *n*-6 eventually accumulated in the HRA biomarker of tissue HUFA proportions [49]. Typical American daily food choices have average scores around −6 to −7, whereas average scores may be near −3 for Mediterranean groups, near +1 for traditional Japanese, and near +3 for traditional Greenland Inuits [49]. The HRA biomarkers of tissue HUFA status for these groups are near 78%, 63%, 40% and 28% of *n*-6 in HUFA, respectively. Gas chromatographic analyses [10,42] allow easy monitoring of the tissue HUFA proportions to compare with observed health status.

7.1. Differences in "Western" and "Mediterranean" diets

The USDA formed a list of "*Key Foods*" [50] with 538 rank-ordered foods consumed by Americans during 2007–2008. The Omega 3–6 Balance Scores [49] for the top 100 foods ranged from +5 to −50 with an un-weighted average near −6, equivalent to a tissue HUFA status near 78% *n*-6 in HUFA. The simple step of deleting ten food items with the most negative Scores gave 90 remaining items with an un-weighted average near −3. The removed items were: soybean oil, −50; mayonnaise, −46; tub margarine, −39; microwave popcorn, −37; "*Italian*" salad dressing, −35; potato chips, −29; stick margarine, −28; vegetable shortening, −28; peanut butter, −24; tortilla chip snacks, −24. Deleting these foods not traditionally present in Mediterranean meals changed the American "*Key Foods*" list to one that fits closer to a "*Mediterranean diet*". Conversely, adding these items to a Mediterranean diet would "*Westernize*" it in a way that has been happening gradually in Mediterranean regions.

The −35 Score for the "*Italian*" salad dressing is much more negative than the −10 for olive oil, suggesting that the dressing used in the USA had oil with more *n*-6 linoleate than is traditional for Mediterranean foods. Importantly, Mediterranean meals traditionally include some seafood items, whereas there were none in the top 100 foods of Americans. The 151 seafoods obtained from the USDA Nutrient Database had an average score of +30 [49]. Adding some seafood items to the 90 remaining food items would give a more positive average value. Explicit information on the 3–6 balance of each food item gives an easy way for people to practice personal primary prevention. The information can be downloaded to personal portable devices to serve as an "app" [51] and guide personal food choices when shopping, preparing meals or discussing foods with friends.

A simplified example in Table 1 illustrates the use of Omega 3–6 Balance Scores: 19 food items combined in a daily menu plan with 100 Cal of each food have an overall Score of +2, which is predicted to maintain a tissue HUFA balance near 35% *n*-6 (or 65% *n*-3) in HUFA. This predicted HRA value resembles that for Greenland Inuits [49] and is associated with a low incidence of cardiovascular disease.

8. Arithmetic for food energy density in health and disease

The 2008 critique of paradoxes in advice on dietary lipids [10] reviewed misunderstandings developed from epidemiological associations and fragmentary evidence about the molecular events by which food energy density has a fatal impact on human health. Biomarkers associated with an imbalance in the intake and expenditure of food energy are: food energy density, dietary fat, dietary saturated fat, plasma triacylglycerols, obesity (body mass index), plasma LDL, plasma cholesterol, insulin resistance and type 2 diabetes. All of these factors predict to some degree a higher risk for

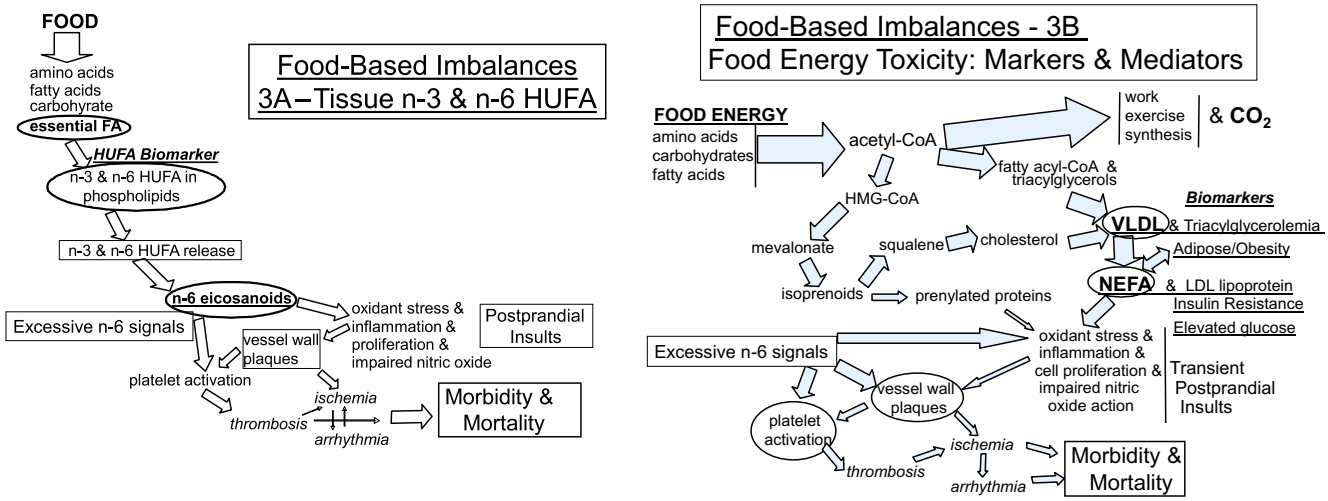

Fig. 3. Two food-based imbalances and their consequences. The Figure shows only a few selected paths and intermediates to help readers focus on causal mediators by which two preventable food imbalances increase morbidity and mortality. Ovals enclose causal mediators for harmful conditions enclosed in rectangles. (A) Imbalanced intakes of *n*-3 and *n*-6 essential fatty acids (EFA) create imbalanced proportions of *n*-3 and *n*-6 in the HUFA of tissue phospholipids. The %*n*-6 in the HUFA of whole blood is a convenient health risk assessment (HRA) biomarker. Low proportions of *n*-3 HUFA mean high proportions of *n*-6 in HUFA that can amplify transient postprandial endothelial dysfunctions into chronic inflammatory plaques and promote ischemia, thrombosis and heart attack. (B) High food energy density tends to imbalance the intake and expenditure of energy, creating transient NEFA-induced endothelial dysfunctions that can be converted more rapidly into chronic inflammatory sites when people have a high %*n*-6 in HUFA.

CVD events even though they may not cause the clinical endpoints of cardiovascular events and death [40].

Fig. 3 helps identify biomarkers that have a causal mediating role in CVD. Only causal mediators are valid surrogates for clinical events during prevention interventions [10]. While treatment medicine may be funded to reduce any unwanted sign or symptom (risk factor), preventive medicine must put a high priority on reducing causal mediators if it is to prevent the need for treatment. Reducing a non-mediating predictive risk factor may still leave the initial cause unchanged to continue creating harm and thereby not reduce the incidence of the disease that must then be treated.

The 2008 critique of advice on dietary lipids described how general understanding of causal fatal mechanisms for saturated fats and cholesterol was incomplete [10]. It described the role of *n*-6-eicosanoids in atherogenesis and confirmed a detailed 1992 review [52] about weaknesses in the cholesterol-oriented "*lipid hypothesis*". Limited logic by advisory groups continues to promote evidence-based misunderstanding of cholesterol actions and leads to interventions which are not cost-effective. More rigorous logic is needed to identify and prevent the mediators by which food energy impairs the important clinical outcomes of saved lives and prevented disease.

A steady, inexorable accumulation of vascular damage after adolescence was confirmed by extensive detailed histology in the PDAY study (reviewed in Fig. 14, Ref. [10]). The area of abdominal aortal damage was near 20% at 18 years, 32% at 28 years and 40% at 32 years. The results show that continual (but infrequent) initiation of vascular damage proceeds for decades unrecognized until the accumulated damage eventually causes a severe clinical event.

Vascular injury begins selectively where hydrodynamics cause eddy currents with extended residence times [53]. These eddies allow accumulated oxidants and inflammatory mediators to amplify a transient vascular dysfunction into a chronic vascular pathology. Once formed, an inflammatory site makes and releases more chemotactic and inflammatory mediators and progresses to even more severe pathology. One potent force in this progression (especially in people with HRA values above 50% *n*-6 in HUFA) is the much greater chemotactic and inflammatory action of *n*-6 LTB4 compared to that for *n*-3 LTB5 [54].

A likely candidate for a very early step in atherogenesis is the repeated postprandial reversible loss of endothelial function [55,56] which could occasionally convert into a chronic inflammatory locus. Endothelium-dependent dilation is lower with higher postprandial triacylglycerolemia (a marker for high food energy density). An often-neglected postprandial process when excess food energy forms the much-discussed circulating blood biomarker low-density lipoprotein (LDL) is the hydrolytic release of large amounts of non-esterified fatty acid (NEFA) into the plasma [10]. The biological impact of the much-neglected NEFA and its resultant oxidant stress (indicated in Fig. 3B) may be greater than the effect of the co-produced LDL (with its adherent cholesterol). However, daily messages about LDL cholesterol from marketing and research groups greatly exceed information on the simultaneously released NEFA, and they divert attention away from harmful NEFA actions.

8.1. Actions of non-esterified fatty acids (NEFA) amplified by n-6 mediators

Meal-induced vascular dysfunction and oxidative inflammatory conditions (measured by hydrogen peroxide and isoprostane levels) as well as released monocyte chemoattractant protein-1 were less when diets included fish oil *n*-3 HUFA [57]. Lipemia-induced loss of endothelial function involves impaired nitric oxide actions, and it can be alleviated in part by supplements of arginine [58]. However, arginine did not prevent an accompanying pro-thrombotic expression of P-selectin and vonWillebrand factor on platelets. Impaired endothelial function monitored as flow-mediated dilation after an oral fat challenge was related to the extent of hypertriacylglycerolemia and oxygen-derived free radicals [59]. Postprandial lipemia was accompanied by increased plasma hydroperoxides and a neutrophil chemotactic agent, IL-8 [60].

Importantly, leukocyte chemotaxis and adhesion are much greater when the mediator is *n*-6 LTB4 rather than *n*-3 LTB5 [54]. A significant increase in adhesion of monocytes to the endothelial monolayer occurred in the presence 20:4*n*-6, and it was decreased with 20:5*n*-3 [61]. Pro-inflammatory mediators (intercellular adhesion molecule 1, vascular cell adhesion molecule 1, E-Selectin, IL-6, and TNFα) were all significantly increased in endothelial cells

incubated with 20:4*n*-6. Thus, the *n*-3 and *n*-6 HUFA proportions in tissues shown in Fig. 3A must be considered when interpreting the impact of food energy density upon risk for CVD shown in Fig. 3B.

Important arithmetic in managing food energy is in balancing intake with expenditure during the course of a day. The on-going societal shift toward a sedentary lifestyle puts a premium on awareness of energy intake and expenditure. During 3 h of typical modern lifestyle activities, a 150 lb person may expend approximately: 225 Cal riding in a car; 202 Cal using computer/internet; 216 Cal watching television; 202 Cal reading book/newspaper; 202 Cal sleeping. Physical activity like walking for 1 h may expend about 270 Cal, and one hour of bicycling, about 500 Cal.

In contrast to low energy expenditure, an average restaurant meal may have 1327 Cal [62], which is 1100 in excess of that likely to be burned in the next 3 h. As a result, much remains for the liver to convert to plasma VLDL and begin the transient process of postprandial endothelial dysfunction. An important, simple tactic to distribute food energy intake more evenly is to eat fewer calories per meal and use small snacks to lower the burden of food energy per hour upon the liver.

With three meals per day and 365 days per year, people may have a thousand postprandial situations per year. If only one per hundred (1%) of these transient postprandial insults converted to a chronic inflammatory site, there might be 10 new sites each year leading to 200 sites in 20 year-old individuals, 400 in 40-year olds and 600 in 60-year olds. Such a low frequency for initiation fits the slow age-dependent histological evidence in the PDAY Study (see Fig. 14 in [10]). While food energy can give reversible pathologies, a more serious process may be the *n*-6 mediated amplification of transient dysfunction into chronic inflammatory plaques.

The propensity for recruiting macrophages that convert a vascular area into a chronic inflammatory site is much greater when the tissue HUFA balance has a high %*n*-6 in HUFA. In this way, the higher risk of mortality associated with higher levels of the food energy biomarker, cholesterol (Fig. 1), is seen in populations that have a higher HRA value for the %*n*-6 in HUFA. A high prevalence of CVD for Americans has remained for decades near 40% for 40-year olds, 60% for 60-year olds and 80% for 80-year olds [63] indicating a failure to prevent the continual disease progression that the PDAY Study showed to begin youth.

Fig. 3A shows how *n*-3 and *n*-6 mediators act in CVD, and Fig. 3B shows how food energy intake leads to a high body mass index (BMI) or obesity, which is a predictive associated risk factor for CVD. While factors that cause obesity may also cause CVD, a high BMI *per se* is not a certain cause of vascular damage, CVD or death. A very large expensive effort to lower CVD by lowering BMI with intensive lifestyle intervention of 5145 overweight or obese patients in 16 study centers [64] gave weight loss through decreased caloric intake and increased physical activity. However, the trial was stopped after millions of dollars and 9.6 years of follow-up showed no lowering of observed risk of cardiovascular morbidity or mortality compared with controls. While many people believe that obesity (high BMI) causes death, the fatal mechanisms and mediators will need to be better identified and prevented if we are to design cost-effective interventions that prevent harm from food energy.

9. Review of advice on dietary lipids

During the past fifty years, the American public has experienced vigorous promotion of the "*cholesterol hypothesis*" that blood cholesterol was a mediating cause of cardiovascular disease. Widespread advice was designed to increase intakes of linoleate and decrease intakes of dietary saturated fats and cholesterol while many drugs were marketed intensively to slow the conversion of food energy to cholesterol. Advice from the FNB noted that *"A Tolerable Upper Intake Level is not set for cholesterol because any incremental increase in cholesterol intake increases CHD risk"* (Ref. [1], p. 542 and 573). This comment indicates a belief in a very narrow to non-existent therapeutic window for dietary cholesterol.

The report also claimed: "*The main adverse effect of dietary cholesterol is increased serum LDL cholesterol concentration, which would be predicted to result in increased risk for CHD*." (Ref. [1], p. 568) The stated rationale for this advice used associative rather than causal terminology: "*There is much evidence to indicate a positive linear trend between cholesterol intake and low density lipoprotein cholesterol concentration, and therefore increased risk of coronary heart disease*" (Ref. [1], p.542 and 573).

In contrast to this interpretation, Fernandez [65] summarized epidemiological studies from the past 20 years that "*show no evidence of a link between dietary cholesterol and heart disease, coronary heart deaths or plasma cholesterol concentrations*." and concluded that "*recommendations limiting dietary cholesterol should be reconsidered*." This view follows from data collected from 37,851 men and 80,082 women which gave no evidence of a significant association between egg consumption and risk of CHD or stroke in either men or women [66].

Despite repeated affirmations that associations are not proof of cause [29], epidemiological studies often monitor surrogate biomarkers that are predictive "*risk factors*" without evidence that the factor actually causes the harmful clinical event. Nevertheless, predictive associated risk factors are often used to "*explain*" differences in death rates [34] and allocate the "*risk due to*" such factors in ways that mislead the public with evidence-based misunderstandings regarding associated and causal factors. The hypothesis of cholesterol-mediated deaths was dominant for decades although extensive associative correlations were not matched with proved causal mechanisms [10,52]. When a large clinical trial, ENHANCE, lowered blood cholesterol without lowering coronary heart disease (CHD) clinical events [67], public questions rose as to whether cholesterol is a valid surrogate marker for preventing CHD [68,69].

The JUPITER trial [70] showed that statin treatment lowered elevated levels of an acute stress protein that is released during inflammatory conditions while it was also lowering plasma cholesterol levels. The results re-opened long-standing questions of whether cholesterol [29] or inflammation [71] is a more important preventable mediator of CVD morbidity and mortality. Fig. 3B notes that isoprenoid molecules other than cholesterol (e.g., prenylated proteins) may mediate inflammatory-proliferative events. However, the transient news report of a single scientific study is easily forgotten as a stream of redundant marketing messages diverts attention. Historic aspects and misunderstandings surrounding the 1984 Cholesterol Consensus Conference in facilitating statin marketing have already been reviewed in detail [10].

9.1. Shifting attention toward inflammation

Thirty years after the 1984 Cholesterol Consensus Conference (with statin patents expiring), new marketing messages have begun to turn attention away from lowering blood cholesterol levels toward lowering actions of inflammatory mediators. Cholesterol is not an inflammatory mediator, and long-standing evidence-based misunderstandings about its actions in health need correction [10,52]. The basic logic for one predictive risk factor is: (1) where there's smoke, there's fire; (2) waving away smoke will not put out a fire. Now is a good time to re-examine the logic in the advice of the Consensus Development Panel that declared blood cholesterol to be a mediating cause of CVD rather than merely an associated predictive factor [72].

The Sydney Diet Heart Study recently gave a comprehensive description of the effects of dietary linoleic acid on CHD and CVD mortality [73]. The long-neglected evidence shows that substituting *n*-6 linoleate in place of saturated fatty acids decreased blood cholesterol levels as predicted from many metabolic studies. However, that substitution also led to a CVD and CHD mortality above the already high rate for the control group (evidence little noted publicly until now). An updated meta-analysis of the intervention trials that increased dietary linoleic acid to lower the blood cholesterol showed no evidence of cardiovascular benefit [73]. Rather, the findings re-open questions about FNB advice [1] to eat more *n*-6 linoleate to lower the blood cholesterol levels that still remain a popular associative predictor of CVD events. Lowering this associative predictive risk factor does not lower the causal factor of excessive *n*-6 mediator actions, and it has not been very cost-effective in preventing CVD.

The evident risk from tissue HUFA imbalances with HRA values above 50%*n*-6 in HUFA [10,38,45] opens the question of setting a Tolerable Upper Intake Level (UL) for linoleic acid intake. However, the FNB claimed "*There is insufficient evidence to set a UL for n-6 polyunsaturated fatty acids*" (Ref. [1], p. 423). To the contrary, the FNB set for linoleic acid an Adequate Intake (AI) level (which nearly all individuals should be eating) *"based on the median intake in the United States where an n-6 fatty acid deficiency is nonexistent in healthy individuals*"(Ref. [1], p. 423). Advice that leads those in the USA population eating less than the median to eat more has an uncertain logic that needs clarification. Nutrition experts might regard it an ethical obligation to inform vegans, vegetarians and people refusing to eat seafoods (that have high positive Omega 3–6 Balance Scores) about the evidence for a very small (or nonexistent) therapeutic window for *n*-6 nutrients in the absence of *n*-3 nutrients.

The currently recommended AI level for *n*-6 linoleate is far above the known level of 0.5 en% at which EFA deficiency is prevented. It was set by the FNB with the stated belief that "*the AI can provide the beneficial health effects associated with the consumption of linoleic acid*". The unspecified evidence for such associations needs very careful review and interpretation. If the putative benefit is in lowered blood cholesterol levels, then advice to eat more linoleate than needed to prevent EFA signs seems poorly justified.

One in three American deaths is due to cardiovascular disease that has not been prevented [74], and over 70% of Americans over 60 years have cardiovascular disease [63]. With known harmful actions of *n*-6 mediators in CVD, the current USA median intake of *n*-6 linoleate near 7 en% merits much more critical evaluation. Open evaluation and clarification of the FNB advice and its underlying logic could give constructive rephrasing in the context of the historic evidence for an EAR below 0.1 en% and an RDA near 0.5 en% for *n*-6 linoleate intakes.

Advising the public about an intake of *n*-6 nutrients at which harm may be detected can be addressed by using evidence of tissue HUFA proportions and their consequences. However, the traditional approach to setting a UL value is confounded by the inescapable context of interactions between *n*-3 and *n*-6 acids competing for accumulation in tissue phospholipid HUFA. No single dietary EFA controls the balance in tissue HUFA proportions that are an important HRA measure. Rather, the proportions are maintained by predictable competitive metabolic interactions among dietary 18-, 20- and 22-carbon *n*-3 and *n*-6 EFA [27,28,41,49]. Without competing *n*-3 nutrients in the diet, even 0.5 en% linoleate (an RDA-like level) will give a tissue HUFA balance with more than 50% *n*-6 in HUFA – a value associated with more harmful long-term health outcomes than with values below 50% [10,38,45].

A tolerable level for *n*-6 nutrient intake needs to be seen in the context of desired long-term outcomes. It will depend on the average daily supply of beneficially competing *n*-3 nutrients that lower the proportion of *n*-6 in HUFA and widen the therapeutic window for dietary linoleate. The multiplicity of successful combinations of *n*-3 and *n*-6 nutrient intakes that can maintain a desired HRA value should not be a reason to limit discussion of a desirable upper limit in the %*n*-6 in HUFA. Precise longitudinal evidence from Collins et al. [13] indicated that a rescue from inadequate to adequate EFA status occurred in going from 30% *n*-6 in HUFA to 50% *n*-6 in HUFA (when the other main tissue HUFA was 20:3*n*-9). Paradoxically, cross-national evidence suggests that long-term harm may occur when going from a balance of 30% to 50% *n*-6 in HUFA (when the other main HUFA are 20:5*n*-3 and 22:6*n*-3). In fact, the multiethnic evidence for progressively less harm with progressively lower HRA values of %*n*-6 in HUFA has no clear "optimum" [38].

9.2. Recognizing unbalanced n-6 nutrient supplies

The biomedical community can constructively consider that essential *n*-6 nutrients may have a much narrower therapeutic window than is generally recognized. The absence of a committee-assigned tolerable upper limit is not evidence for the absence of a tolerable upper limit. Ironically, FNB advice implying a nonexistent therapeutic window for dietary cholesterol might logically be withdrawn and replaced by advice about a very small to nonexistent therapeutic window for dietary linoleic acid in the absence of counterbalancing *n*-3 nutrients. Lowering the dietary supply of *n*-6 nutrients ensures that counter-balancing *n*-3 HUFA can accumulate in tissues from which they form (often at slower rates) less aggressive mediators. One simple result of accumulating higher proportions of *n*-3 in tissue HUFA is that it lowers the proportion of *n*-6 in HUFA available for release by phospholipase A2, and it slows the formation and action of potent *n*-6 bioactive lipids (as many pharmaceuticals are designed to do). Future research will apportion further the health benefits that may come from lessened actions of *n*-6 mediators compared to increased actions of *n*-3 bioactive lipids (such as resolvins, protectins, maresins and epoxides). Both types of benefit favor lowering dietary intakes of *n*-6 nutrients while raising intake of *n*-3 nutrients.

A study of worldwide diversity in disease burdens and intakes of EFA led to estimates of how much added dietary *n*-3 HUFA could give different groups a target HRA status of 50% *n*-6 in HUFA [75]. Because of very different intakes of *n*-6 linoleate, that HRA goal might require added intakes of 1 en% *n*-3 HUFA for the USA, 0.5 en% for Italy, 0.26 en% for Denmark and only 0.06 en% for the Philippines. The report [75] estimated that a healthy dietary allowance for *n*-3 HUFA with current US diets could be 3.5 g/d for a 2000-kcal diet, and it *"can likely be reduced to one-tenth of that amount by consuming fewer n-6 fats"*.

Measuring only *n*-3 status or *n*-6 status alone fails to keep the important context of balance between *n*-3 and *n*-6 nutrients and tissue HUFA balance that underlies health maintenance and disease prevention. In this regard, rather than elevating intakes of *n*-3 HUFA to 3.5 g/d to achieve an HRA status of 50%, the average American diet could be adjusted by lowering *n*-6 linoleate intake from its current level near 16.5 g/d (6.8 en%) to a level near 2.5 g/d (1en%). The diet-tissue estimator [41] shows that if intake of *n*-3 linolenate remained at 0.7 en%, lowering *n*-6 linoleate intake to 1 en% for Americans could meet an HRA goal near 50% *n*-6 in HUFA. If linolenate intake continued to be only 10% of the linoleate intake, then an additional 0.06 en% *n*-3 HUFA could meet the HRA goal of 50%.

As noted earlier [49] with the Omega 3–6 Balance Scores and the American Key Foods list, reducing the linoleate content of the diet is easily achieved by replacing soybean oil (with an omega 3–6 balance score of −50) with an oil of low linoleate content and by eating other food with less negative Omega 3–6 Balance Scores. Olive oil (score of −10) has a lower content of *n*-6 linoleate

than most vegetable oils, but it has very little counterbalancing *n*-3 linolenate. Alternatively, canola oil (score, −11) has twice the content of linoleate as olive oil with some counterbalancing *n*-3 linolenate. Finally, flaxseed oil (score of +48) has *n*-6 linoleate content similar to canola counterbalanced by 3-fold more *n*-3 linolenate.

Various cultivars and genetically modified plant oils that can help people lower the %*n*-6 in HUFA are now approaching public markets. At present, one readily available low-linoleate food-oil is from the high-oleic acid cultivar of sunflower (score of −4; which contrasts with −74 for standard sunflower oil). Recently, food combinations [76] that comply with the official Australian Guide to Healthy Eating were made with a *n*-6 linoleate daily intake near 1.8 en% by using macadamia oil (score, 0) and butter (score, −3). Also, a highly successful nutritional intervention to reduce severe headaches [77] used diets made with study-provided low-linoleate oils such as coconut oil (score, −2), macadamia nut oil (score, 0), butter (score −3), fat-free mayonnaise and macadamia-vinaigrette salad dressing [78].

Lowering intakes of linoleate to less than 2.5 en% allowed an increased intake of omega-3 nutrients for three months to replace tissue *n*-6 HUFA with *n*-3 HUFA and lower the HRA status from 77 to 61%*n*-6 in HUFA while achieving an intended lowering of clinical signs and symptoms [70]. Fortunately, the less vigorous actions of *n*-3 nutrients have no known UL [79], and their competitive actions widen the narrow therapeutic window for *n*-6 nutrients. Overall evidence supports advising an estimated average requirement (EAR) for *n*-6 linoleate near 0.1 en%, a recommended dietary allowance (RDA) near 0.5 en% and a tolerable upper intake level (UL) near 2 en%. Similar values for an EAR and RDA are appropriate for *n*-3 linolenate. Such advice can help more people eat foods that may lower the prevalence of many chronic immune-inflammatory-thrombotic disorders.

10. Follow the money

The 2008 critique of paradoxes in advice on dietary lipids described how "silo mentality" [10] among diverse special interest groups gave the public fragmented facts that led to serious evidence-based misunderstandings. However, the review failed to give an adequate analysis of the financial incentives that could permit progress in primary prevention. The review made it clear that business plans for food processing and marketing have financial imperatives very different from preventive medicine. Evidence for a narrow therapeutic window for dietary linoleic acid (without counterbalancing *n*-3 nutrients) may not be a welcome addition to messages for marketing most vegetable oils and nuts, even though it might fit marketing messages from developers of new low-linoleate food oils.

In a similar way, the prevention of a need to treat may not be a welcome idea for the many people gainfully employed in treatment-oriented activities: doctors, nurses, actuaries, researchers, health care professionals and wellness counselors working with hospitals, pharmaceutical companies and insurers. The American health care system of payments offers little financial incentive to professionals who prevent the need for their services. Successful prevention will require explicit identification of preventable causal mediators to allow decisive action by people who will gain financially from removing the cause of the need for treatments. When we identify those who can gain financially from preventing a need for treatments, we likely will learn which people are motivated to support and implement cost-effective prevention.

Twenty years ago, well-informed medical experts noted that "If there were no illness and no accidents, health care costs for a society would theoretically be zero" and "Preventable illness makes up approximately 70 percent of the burden of illness and the associated costs" [80]. An alternate pessimistic review of workplace wellness programs [81] confirmed that not all efforts at prevention save money [80]. Other health specialists have scoffed at the idea that food-oriented interventions might give any appreciable benefit [82]. This attitude is supported by awareness of the imprecise tools of traditional diet assessment and extensive evidence of poor compliance with nutritional interventions. However, these weaknesses are likely less when using a HRA biomarker that quantitatively monitors EFA intakes and also has a mediating role in clinical processes we want to prevent. This was most recently seen for positive benefits in the randomized controlled trial of pain reduction in which patients ate foods that lowered the average HRA value from 77 to 61%*n*-6 in HUFA in three months [77].

Fig. 3 gives a context to see how reducing associated predictive risk factors without removing the primary cause can be expected to be less cost-effective than reducing risk factors that have clear causal connections. The recent disappointing lack of CVD prevention from hopeful reductions in obesity [64] is mirrored in an equally disappointing financial analysis of weight-loss' inability to lower health expenditures [83]. Parallel disappointments occurred when lowering elevated levels of the associated biomarkers, cholesterol and glucose, noted in Fig. 3B (and reviewed in Ref. [40]). Until now, the health care plans developed by treatment-oriented health professionals have not implemented cost-effective prevention interventions even though those professionals remain gainfully employed from their treatment-centered actions.

10.1. Who has health-related financial losses?

With health-related absenteeism and presenteeism causing much more financial loss than their associated medical and pharmacy claims, self-insured corporations have many financial reasons to prevent the many health-related problems associated with high proportions of *n*-6 in HUFA and excessive *n*-6 mediator actions. Such problems, made worse by uninformed food choices, include heart attacks, atherosclerosis, thrombosis, arrhythmia, stroke, immune-inflammatory disorders, asthma, arthritis, cancer proliferation, obesity, psychiatric disorders, depression, suicide, homicide, oppositional behavior, unproductive workplace behaviors and length of stay in hospitals. A financial loss for health-related absenteeism and presenteeism plus medical and pharmacy costs would not occur for healthy employees.

Being aware of this, most large employers already invest in corporate wellness plans to help employees build healthy lifestyles. Successful plans will give an objective measure of return on investment. People do not need a physician's prescription to eat less *n*-6 nutrients, more *n*-3 nutrients and fewer calories per meal. Each corporate wellness plan that offers employees explicit information on omega 3–6 balance scores of foods plus results from their personal HRA monitoring can generate its own informative summary of anonymous individual associations of HRA values of %*n*-6 in HUFA linked to annual health care claim costs. Fragmentary results suggest that employees who maintain lower HRA values may have lower average annual health claim costs [84].

With a clear focus on preventing financial losses due to an unintended high HRA status, two tools give a new opportunity for corporations to inform their employees of ways to shift attention away from non-causal biomarkers toward effective prevention [84,85]. The %*n*-6 in HUFA measured by gas chromatographic analysis of HUFA in an individual's finger-tip blood-spot readily informs people of their HRA status [42]. Knowing one's HRA value and its consequences can motivate voluntary use of Omega 3–6 Balance Scores [49,51] to choose foods with less negative and more positive Scores.

Voluntary informed choices of foods that shift tissue HUFA balance and lower the average HRA status of employees from 77% *n*-6

in HUFA to 57% *n*-6 in HUFA might prevent more than $400 million financial losses annually for a self-insured corporation with 100,000 employees and a typical health-care plan [84]. In the USA overall, there may be a trillion dollars of preventable annual financial loss that self-insured corporations could recover and redirect to other priorities. The evidence examined in this review suggests that employees and employers in the USA have much to gain together from monitoring and preventing imbalances among *n*-3 and *n*-6 hormone precursors.

We know of tools ready to monitor [42] and moderate [49,51] imbalances in *n*-3 and *n*-6 nutrients that cause hundreds of billions in corporate financial losses in the USA. The FNB quoted Goethe when advising the public about DRI values [1]: "Knowing is not enough; we must apply. Willing is not enough; we must do."

Conflict of Interest

The author declares that there are no conflicts of interest.

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